Opis delovnega mesta mladega raziskovalca/ke (D*escription of the Young Researcher's position)*

1. Članica UL (*UL member)*:

Biotehniška fakulteta / Biotechnical faculty

2. Ime, priimek in elektronski naslov mentorja/ice (*Mentor's name, surname and email*):

Kristina Sepčić (kristina.sepcic@bf.uni-lj.si)

3. Raziskovalno področje (*Research field*):

Biokemija i molekularna biologija / Biochemistrty and molecular biology

4. Opis delovnega mesta mladega raziskovalca/ke (D*escription of the Young Researcher's position)*:
Vključuje morebitne dodatne pogoje, ki jih mora izpolnjevati kandidat/ka za mladega raziskovalca/ko, ki niso navedeni v razpisu za mlade raziskovalce.

Mladi raziskovalec se bo vključil v raziskovalno delo programske skupine Toksini in biomembrane (P1-0207) in Skupine za biokemijo (0481-204), ki se med drugim ukvarja s proučevanjem beljakovin, ki se vežejo z biološkimi ter umetnimi lipidnimi membranami, jih preoblikujejo in v njih tvorijo transmembranske pore. V zvezi s temi procesi preučujemo potencialno uporabo teh beljakovin v biomedicini, farmaciji in drugje. Mladi raziskovalec se bo ukvarjal s proteini iz družine egerolizinov, ki jih proizvaja užitna goba bukov ostrigar. Omenjeni egerolizini specifično interagirajo z membranskimi domenami, obogatenimi s sfingomielinom in holesterolom, zato se lahko uporabljajo kot ne-toksični označevalci za vizualizacijo strukture in dinamike membranskih raftov v živih celicah. V kombinaciji s partnerskimi proteini, ki vsebujejo domeno MACPF (domena, ki napade membrano/perforin), egerolizini v tarčni membrani tvorijo bikomponentne transmembranske pore. V preteklosti smo pokazali, da nativni izolat iz bukovega ostrigarja, ki vsebuje mešanico egerolizina OlyA6 in MACPF proteina PlyB, specifično uničuje urotelijske rakave celice, ki imajo v membranah povečano število membranskih raftov. Nedavno pa smo v skupini pripravili nabor novih rekombinantnih egerolizinskih proteinov in njihovih mutiranih različic, kakor tudi njihovih partnerjev z domeno MACPF. Vsi omenjeni proteini imajo izboljšane lastnosti (ojačano interakcijo z membrano in bolj učinkovito spodobnost njene permeabilizacije). Razen tega so nedavno odkrili, da se določeni egerolizini iz ostrigarjev, ki ne interagirajo z lipidnimi rafti, vežejo tudi na membranski lipid kardiolipin. Kardiolipin se v apoptotskih celicah prestavi iz notranje mitohondrijske membrane v zunanjo in od tod na plazmalemo, zato je lahko uporaben kot biomarker apoptoze. Mladi raziskovalec bo v okviru usposabljanja ovrednotil: (i) potencial fluorescenčno označenih izbranih egerolizinov in njihovih mutiranih različic kot označevalcev urotelijskih rakavih celic; (ii) potencial kompleksov izbranih egerolizinov iz točke (i) in njihovih proteinskih partnerjev z domeno MACPF za selektivno uničevanje celic raka urotelija;

(iii) potencial izbranih egerolizinov, ki interagirajo s kardiolipinom, kot biomarkerjev apoptoze.

Delo bo obsegalo:
(i) pripravo in izolacijo rekombinantnih egerolizinskih in MACPF-proteinov v bakterijah, pripravo umetnih lipidnih sistemov (lipidnih veziklov), ki vsebujejo ustrezno lipidno tarčo;
(ii) vrednotenje vezave (test sedimentacije, površinska plazmonska resonanca) in permeabilizacije umetnih lipidnih membran (fluorimetrični test sproščanja kalceina) z egerolizini in egerolizinskimi kompleksi, ter analizo strukture membransko vezanih proteinov s krio-elektronsko mikroskopijo;
(iii) vrednotenje interakcije egerolizinov in egerolizinskih kompleksov z biološkimi membranami – netransformiranimi normalnimi in rakavimi urotelijskimi celicami v fizioloških pogojih ter po indukciji apoptoze (vrednotenje vezave fluorescenčno označenih egerolizinov s konfokalno mikroskopijo, testi citotoksičnosti z merjenjem luminiscence na mikrotitrskih ploščah in s pretočno citometrijo).
Poskusi s celičnimi kulturami urotelija bodo potekali na Inštitutu za biologijo celice Medicinske fakultete v Ljubljani.

Mladi raziskovalec se bo vpisal na interdisciplinarni podiplomski študij Biomedicine – smer Biokemija in molekularna biologija. Zaželeno je, da ima kandidat izobrazbo iz področja bioloških ved, predvsem iz področja molekularne biologije in biokemije ter izkušnje z laboratorijskim delom. Zaželeno je tudi dobro znanje angleškega jezika in programskega orodja Microsoft Office.

The young researcher will participate in the ongoing research activities of the Research program P1-0207 (Toxins and biomembranes) and the Research group of biochemistry (0481-204). These groups focus on research of proteins that interact with biological and artificial lipid membranes, form transmembrane pores and/or remodel these membranes. We are particularly interested in potential use of these proteins in biomedicine, pharmacy and other fields.

Within her/his PhD thesis, the young researcher will study membrane interactions and potential applications of aegerolysin proteins from the edible oyster mushroom. These proteins specifically interact with membrane microdomains enriched in sphingomyelin and cholesterol, and thus can be used as non-toxic markers for studying the structure and dynamics of membrane rafts in living cells. In concert with their partnering proteins bearing the membrane-attack domain/perforin (MACPF) domain, aegerolysins can form bicomponent transmembrane pores in target membranes. We showed that the native oyster mushroom isolate, containing the mixture of the aegerolysin protein OlyA6 and its MACPF-partnering protein PlyB, specifically eliminates urothelial cancer cells whose membranes are enriched in lipid rafts. We have recently isolated a number of novel recombinant aegerolysins and their mutants, as well as their partnering MACPF-proteins. All these proteins have improved characteristics (enhanced membrane binding and more effective membrane permeabilization). Besides that, it was recently discovered that selected aegerolysins from oyster mushroom do not interact with lipid rafts, but with another membrane lipid – the cardiolipin. During the apoptosis, this lipid translocates from inner to the outer mitochondrial membrane and finally reaches the plasmalemma; therefore, it can be used as a biomarker of apoptosis.

Within her/his PhD thesis, the young researcher will evaluate:
(i) the potential of the fluorescently labelled selected aegerolysins, and their mutated versions, for labelling urothelial cancer cell membranes;
(ii) the potential of the complexes of selected aegerolysins (from point (i)) and their partnering MACPF-proteins to selectively eliminate urothelial cancer cells;

(iii) the potential of selected cardiolipin-binding aegerolysins as biomarkers of apoptosis.

The work will comprise:
(i) Preparation and isolation of recombinant aegerolysins and their MACPF-partnering proteins in bacteria, preparation of artificial lipid systems (lipid vesicles) containing appropriate target lipids;
(ii) Evaluation of protein interaction with model lipid membranes (using sedimentation test, surface plasmon resonance, fluorimetric calcein-release test); analysis of the structure of proteolipid complexes using cryo-electron microscopy;
(iii) Evaluation of the interaction of aegerolysins and aegerolysin-based complexes with biological membranes – non-transformed normal and cancer urothelial cells in physiological conditions and after induction of apoptosis (evaluation of the binding of fluorescently labelled aegerolysins using confocal microscopy, cytotoxicity luminescence tests on microtiter plates and cytotoxicity evaluation using flow cytometry).
The experiments with urothelial cell lines will be performed at the Institute of Cell Biology, Medical faculty in Ljubljana.

The young researcher will attend the interdisciplinary doctoral study in Biomedicine (course: Biochemistry and molecular biology). A graduate degree in the field of biological sciences, particularly in molecular biology and biochemistry, is preferred. Experience with laboratory work and a good knowledge of English language and Microsoft Office is desirable.